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8   **Abstract**

9   The objective of this study was to evaluate the effects of age on growth and fecal parameters,

10   nutrient digestibility, hematology, and serum biochemistry of Beagle dogs. A total of 18 healthy Beagles

11   were allotted into three groups according to age (puppy, under 1 year old; adult, 1–7 years old; senior, over

12   7 years old). The study lasted for 17 days, with a 7-day adaptation period and 10-day evaluation period.

13   During the evaluation period, individual body weight, length, and food intake were estimated and recorded

14   to calculate growth parameters. Fecal samples were collected from all beagles to evaluate fecal score and

15   nutrient digestibility by visual observation and chemical analyses, respectively. Blood samples were

16   collected from each dog to evaluate hematological and biochemical parameters using automated

17   hematology analyzer and clinical autoanalyzer, respectively. Puppy group had increased ($p < 0.05$) body

18   weight change during the study compared with adult or senior groups. At the end of the food trial, body

19   condition score of all age groups were ideal. Puppies had higher ($p < 0.05$) fecal moisture content and

20   diarrhea frequency than adult or senior dogs. Senior group had lower ($p < 0.05$) apparent total tract

21   digestibility (ATTD) of crude protein than puppy or adult groups. Puppy group had lower ($p < 0.05$) ATTD

22   of ether extract, crude fiber, and nitrogen-free extract than adult or senior groups. Puppies had higher

23   number of white blood cells and neutrophils at the initial ($p < 0.05$) and final ($p = 0.062$) day of the

24   evaluation period than senior dogs. Senior dogs had lower ($p < 0.05$) levels of serum albumin, glucose, and

25   creatinine during the evaluation than puppies. Puppy group had higher ($p < 0.05$) serum alkaline phosphate

26   level at the initial and final day of the evaluation than adult or senior groups. In conclusion, our study

27   determined the effect of age on growth and fecal parameters, nutrient digestibility, hematology, and serum

28   biochemistry of Beagle dogs.

29

Introduction

31

32   Companion animals are considered family members who share our lives. Changes in population

33 structure, socioeconomic development, and cultural changes have led to an increase in companion animal

34 ownership worldwide, leading to greater interest in their health and well-being [1–4]. People are

35 increasingly concerned about the quality of the foods for their companion animals, including protein sources,

36 and are investing generously in high-quality and functional products to promote animal health [4,5]. In

37 addition, as the lifespan of companion dogs has increased due to improved veterinary care and quality of

38 canine foods, it is important to understand the physiological changes for nutritional management throughout

39 their life cycle [6,7]. As a result, academic efforts to optimize nutrient utilization and promote health

40 throughout the lifespan of dogs are becoming increasingly important. For this purpose, several

41 organizations such as the National Research Council (NRC), the Association of American Feed Control

42 Officials (AAFCO), and the European Pet Food Industry Federation (FEDIAF) have presented

43 recommendations for nutrient profiles for dogs [8–10]. However, these standards may have limitations in

44 reflecting the diversity of all breeds and ages, and the results of previous studies on nutrient utilization and

45 health are diverse. Thus, it is necessary to investigate age-related physiological changes through various

46 studies.

47   Nutritional physiology of companion dogs undergoes dynamic changes throughout their life stages.

48 In particular, during the growth period, high nutrient requirements are needed due to tissue development,

49 skeletal formation, and immune system maturation [11–14]. On the other hand, during the aging period,

50 appropriate nutritional management is essential for maintaining health due to decreased digestive efficiency,

51 altered gut microbiota, and reduced immune functions [13,15,16]. Thus, aging is a key variable that

52 influences the digestive function, nutrient metabolism, immune responses, and tissue functions. Previous

53 studies on improving the health of companion dogs have evaluated various indicators such as nutrient

54 digestibility, hematological and biochemical parameters, and gut microbial changes, however these studies

55 focused on one or two age groups [7,17,18]. Therefore, research on a comprehensive approach is needed to

56 characterize physiological changes by age. The present study hypothesized that there would be changes in

growth and fecal characteristics, nutrient digestibility, and hematological and biochemical indices of dogs with age, even when fed same canine foods. Physiological differences are an essential consideration for food formulation and age-specific health management. Therefore, this study aimed to evaluate growth and fecal parameters, nutrient digestibility, hematology, and serum biochemistry of three life stages of Beagle dogs fed the same diet.

Materials and Methods

Animal ethics

The protocol for this study was reviewed and approved by the Institutional Animal Care and Use Committee of Chungnam National University, Daejeon, South Korea (approval #: 202310A-CNU-179).

Experimental design, animals, and diet

Eighteen clinically healthy Beagle dogs were divided into three age groups: puppies (n = 6; under 1 year old; average age of 48.2 ± 11.3 weeks; mean body weight [BW] = 6.21 ± 0.61 kg; 2 neutered males and 4 neutered females), adults (n =6; 1–7 years old; average age of 4.5 ± 2.1 years; mean BW = 8.16 ± 0.70 kg; 3 neutered males and 3 neutered females), and senior dogs (n = 6; older than 7 years; average age of 9.5 ± 0.8 years; mean BW = 6.95 ± 1.52 kg; 3 neutered males and 3 neutered females). Dogs were individually housed in stainless steel cage (0.9 × 0.9 × 1.0 m) with automatically controlled temperature throughout the study (diet adaptation from days -7 to 0; data and sample collection from days 1 to 10). The experimental diet was formulated using hydrolyzed chicken powder, brown rice, and soy protein to meet nutrient profiles for growth and reproduction minimum (crude protein and indispensable amino acids) or adult maintenance minimum (crude fat, calcium [Ca], phosphorus [P], and Ca to P ratio) provided by the Association of American Feed Control Officials (AAFCO) and manufactured in extruded form (Table 1 and 2) [19]. The dog food was provided by calculating maintenance energy requirements (MER) for each dog (puppy, $132 \times BW^{0.75}$; adult, $132 \times BW^{0.75}$; senior, $105 \times BW^{0.75}$) based on standard equations [20]. All dogs were fed twice daily (09:00 and 17:00).

83

Data and sample collection

After the food adaptation period, individual dog BW and body condition score (BCS) were weighed and estimated at the beginning and end of the data and sample collection period and food intake was recorded each day. Body weight change (BWC) and daily food intake (DFI) were calculated for growth parameters of Beagle dogs. The BCS of each dog was measured using a frame marked with the length of chest and abdomen, and the ratio was calculated to evaluate fatness related to health conditions [21]. The BCS scale ranged from 1 to 9 (under BW to over BW), with an ideal score of 4 to 5, according to the American Animal Hospital Association [22]. Blood samples were collected from each dog using 5 mL tubes (BD Vacutainer Systems, Franklin Lakes, NJ, USA) with or without ethylenediaminetetraacetic acid (EDTA) via leg vein on day 1 and 10 after a 12 h fasting period [23]. The collected blood samples in non-EDTA tubes were centrifuged at 3,000 rpm for 15 minutes at 4 °C to obtain serum samples and stored at -80 °C for biochemical analysis [24]. Fecal samples were collected at least twice daily from days 2 to 9 before feeding to evaluate the fecal score and nutrient digestibility using visual observations and total collection method, respectively [25,26]. The fecal score was recorded from 1 to 5 (hard dry to watery diarrhea) with half numerical increments by two independent visual evaluators [27]. The frequency of diarrhea or hard dry feces was calculated by counting cage days with a pen fecal score ≥ 3.5 or < 2.0, respectively. During total fecal collection, feces were collected, weighed, and stored at -80 °C with diet until nutrient digestibility analyses.

Nutrient digestibility analyses

Frozen diet and fecal samples were thawed at room temperature, dried in a forced-air oven (FC-PO-91, LabHouse, Pocheon-si, Gyeonggi-do, South Korea) at 65 °C for 72 hours, and ground using a coffee grinder (Electric Coffee Grinder, Hamilton Beach, USA) for chemical analyses [28]. The ground samples were analyzed for dry matter, energy using a bomb calorimeter (C2000, IKA Works Inc., Staufen, Germany), and crude protein using Kjeldahl method (VAPOXX, Gerhardt Ltd., IdarOberstein, Germany), ether extract, crude fiber, crude ash, nitrogen-free extract (NFE), calcium, and phosphorus according to the

110 Association of Official Analytical Chemists methods [29]. Amino acids contents were analyzed using high-

111 performance liquid chromatography (Waters Alliance System with a Waters 1525 Binary HPLC Pump,

112 Waters 2707 Autosampler, and Waters 2475 Multi λ Fluorescence Detector, Milford, USA) [30]. The

113 analyzed chemical compositions were calculated to determine apparent total tract digestibility (ATTD)

114 according to a previous report [31].

115

116 **Hematological and biochemical analyses**

117 The blood samples collected in tubes containing EDTA were analyzed for complete blood count

118 (CBC) using an automated hematology analyzer (Mindray BC-5000 auto hematology analyzer, Mindray

119 Medical France Sarl, HyTest Ltd, China). The CBC test included fifteen parameters: number of white blood

120 cells (WBC) and their differentiation counts and proportions (neutrophil, lymphocyte, monocyte, and

121 eosinophil) and red blood cell profile (hematocrit, mean cell volume [MCV], mean cell hemoglobin [MCH],

122 mean corpuscular hemoglobin concentration [MCHC], red cell distribution width-coefficient of variation

123 [RDW-CV], red cell distribution width-standard deviation [RDW-SD], platelet, and mean platelet volume

124 [MPV]). Serum samples were analyzed for biochemical parameters using a clinical autoanalyzer (Roche

125 Cobas c 111 chemistry analyzer, Roche Diagnostics Ltd, USA). The serum biochemical test included eleven

126 parameters: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase (ALP), creatinine,

127 calcium, albumin, glucose, total protein, triglyceride, total cholesterol, and blood urea nitrogen.

128

129 **Statistical analyses**

130 Data were analyzed using the GLM procedure of SAS software (v. 9.4; SAS Institute Inc., Cary,

131 NC, USA) in a completely randomized design. Experimental unit was the Beagle dog. Differences in means

132 among treatments were determined using Tukey's multiple range test. Diarrhea and hard dry feces

133 frequency were analyzed using the Chi-square test of SAS. Differences were considered significant at $p <$

134 0.05 and tendency at $p < 0.10$.

135

136 # Results

Growth and fecal parameters

Puppies had increased ($p < 0.05$) BWC during the overall experiment compared with adults or senior beagles (Table 3). The DFI values (as-is, DM basis, calorie basis) were highest ($p < 0.05$) in puppies, followed by adults and senior dogs. In addition, daily fecal output (DFO) values (as-is and DM basis) were highest ($p < 0.05$) in the puppy group, followed by the adult and senior groups. Puppies were higher ($p < 0.05$) calorie-based DFO than adult or senior dogs. However, there was no difference on calorie-based DFO between the adult and senior groups. In fecal characteristics, the puppy group had higher ($p < 0.05$) fecal moisture content than the senior group. Additionally, puppies had higher ($p < 0.05$) diarrhea ratio than adult or senior dogs. However, there were no difference on fecal score and hard dry feces frequency among the age groups.

Nutrient digestibility

The ATTD using the total collection method among age groups are shown in Table 4 and 5. Senior dogs had lower ($p < 0.05$) ATTD of crude protein than the puppies or adults (Table 4). In addition, senior dogs tended to have lower ($p = 0.082$) crude ash digestibility than puppies. The puppy group had lower ($p < 0.05$) ATTD of ether extract, crude fiber, NFE, and phosphorus than the adult or senior groups. The ATTD of calcium was highest ($p < 0.05$) in senior dogs, followed by adult dogs and puppies. However, there were no differences on the ATTD of indispensable and dispensable amino acids among age groups (Table 5).

Hematological and biochemical profiles

The hematological and biochemical profiles are shown in Table 6 and 7, respectively. At the beginning of the food trial, the puppy group had higher ($p < 0.05$) number of WBC than the adult or senior groups (Table 6). In addition, the puppies had higher ($p < 0.05$) number of neutrophils and monocytes than the adult or senior dogs. The MCHC level had highest in the adult group followed by the puppy group and the senior group ($p < 0.05$). On the last day of the study, puppies have tended to higher WBC ($p = 0.062$) and neutrophil ($p = 0.096$) counts than senior dogs. Additionally, the puppy group had higher ($p < 0.05$)

164   lymphocyte counts on day 10 than the adult group. The neutrophil proportion was higher ($p < 0.05$) on day

165   10 in adult dogs than in senior dogs. In the red blood cell profile, the puppy group had higher ($p < 0.05$)

166   MCV level on day 10 than the adult or senior groups. The MCH level showed a higher ($p < 0.05$) in puppies

167   than senior dogs. The adult group tended to have a higher ($p = 0.080$) MCHC level than the senior group.

168   The RDW-CV and RDW-SD values were higher ($p < 0.05$) in senior dogs than adult dogs. The puppy group

169   had lower ($p < 0.05$) MPV level on day 10 than the adult or senior groups.

170   In the serum biochemistry at the start of the study, adult dogs had lower ($p < 0.05$) albumin, glucose,

171   and creatinine levels than puppies or senior dogs (Table 7). A trend was observed in serum triglyceride

172   level ($p = 0.085$), with the senior group have tended to higher than the puppy group. Adult dogs tended to

173   have a higher ($p = 0.072$) the BUN level than puppies. The puppy group had a higher ($p < 0.05$) level of

174   ALP in serum than the adult or senior groups. On the last day of the food trial, senior dogs had lower ($p <$

175   0.05) serum albumin, glucose, and creatinine levels than puppies or adult dogs. However, levels of serum

176   BUN, calcium, and ALP were higher ($p < 0.05$) in the puppy group than the adult or senior groups.

177

178   # Discussion

179   Our study demonstrated that Beagles fed the same diet showed age-related differences in growth

180   and fecal parameters, nutrient digestibility, hematology, and serum biochemistry. These findings reflect

181   physiological and metabolic differences in nutrient utilization according to age and suggest the need for

182   age-related feeding programs in canine food design. During the pre-adult period (< 1 year of age; post-

183   weaning and growing period), MER is high due to high nutritional metabolic demands for growth and body

184   tissue synthesis [20]. On the other hand, during adult and senior periods, food intake is relatively low

185   compared to puppies because it is recommended to meet the MER for maintenance metabolism.

186   Accordingly, the food supply amount in our study was set based on the NRC, and DFI differed among the

187   age groups based on the as-is, DM basis, and calorie basis. In addition, puppies showed the highest BWC,

188   indicating that energy utilization through dog food was effective during the period when energy for growth

189   was required, as mentioned above. Adult and senior dogs with low BWC would have relatively low energy

190 utilization due to a lower food supply compared with puppies, as their nutritional metabolism is focused on

191 maintenance. In particular, the decreased BWC of the senior group indicates that the efficiency of energy

192 metabolism may decrease with age. However, the BCS was remained within the ideal range (score of 4–5)

193 [22] in all age groups at the end of the study suggests that the feeding amount of experimental canine food

194 was appropriately adjusted to the NRC-based recommendations.

195   In fecal parameters, the DFO based on the as-is and DM basis followed the order of the puppy,

196 adult, and senior groups can be seen as the result of "high input, high output" in proportion to the amount

197 of food supply and intake, but there may be differences in energy utilization efficiency among age groups.

198 This is because the DFO based on the as-is and DM basis in the senior group was lower than in the adult

199 group, but there was no difference in calorie-based DFO between the two groups. The fecal moisture content

200 and frequency of diarrhea were higher in puppies. The high diarrhea rate in puppies may be due to

201 underdeveloped gut health, such as an immature digestive system and/or an imbalance in the gut microbial

202 environment at this phase, or may be due to a short gut transit time following high intake. Previous studies

203 have reported that controlling the crude protein content in the feed of weaned piglets can prevent diarrhea

204 caused by protein fermentation [32,33]. In this study, the canine food followed the minimum amount of

205 crude protein for growth and reproduction, including amino acids, and the minimum amount of crude fat,

206 calcium, and phosphorus for adult maintenance. Although the experimental food contained a high level of

207 crude protein compared with the minimum level, which may be associated with diarrhea in puppies with an

208 immature gut system, the growth parameters confirmed that the diet supported a high MER during this

209 period. These findings emphasize the need to provide an appropriate nutritional profile considering age of

210 dogs.

211   Nutrient digestibility is a dynamic physiological response influenced by the utilization of nutrients

212 from food, and the overall health and wellness of companion dogs, and varies according to the age of dogs.

213 From birth to the adult period, the secretion and activity of digestive enzymes develop rapidly [34]. In

214 addition, nutrient digestibility increased with age, but also differed with breed size [35]. Previous studies

215 have reported that nutrient digestibility was stable or even higher rather than decreasing with age

216 [7,13,36,37]. In addition to age, factors that influence nutrient digestibility of dogs include nutrient

217 composition (dietary fiber, starch, and bioactive substances) and ingredients (plant and meat) of the food

218 and the technical manufacturing process (heating, cooking, extrusion, and particle size) [38,39]. For

219 instance, the ATTD of dry matter and main nutrients (crude protein, ether extract, starch, and carbohydrates)

220 in cooked food, including meat (lamb meal, poultry meal, or fish meal) and plant ingredients (wheat and

221 corn-based) is 79–99% [40]. On the other hand, uncooked, unextruded, or overheated ingredients can

222 reduce nutrient digestibility of dogs, especially protein and amino acids [2,41]. Pet food regulatory agencies,

223 including AAFCO and FEDIAF, base minimum nutrient requirements on assumed digestibility of at least

224 70% for dry matter and 80% for protein [42]. Additionally, canine nutritionists generally consider 80% to

225 be an appropriate minimum target for digestibility, with 80-90% being the ideal value for nutrients [42]. In

226 this study, the ATTD of dry matter, organic matter, and energy was 88.95–89.76%, 95.27–96.10%, and

227 92.68–93.51%, respectively, confirming the appropriate basic digestibility among age groups.

228 We determined differences on digestibility of other nutrients by age groups. The minimum

229 recommendation for crude protein is higher during the growth and reproductive stages than during the

230 maintenance period in adults [19]. These differences reflect fundamental biological differences between

231 life stages and are supported by the results of the ATTD of crude protein. Additionally, the decreased ATTD

232 of crude protein in the senior group is considered to be due to complex factors such as reduced gut functions

233 and digestive enzyme secretion and activity following aging. On the other hand, it has been reported that

234 the activities of pepsin, trypsin, and chymotrypsin, which are involved in protein digestion, develop rapidly

235 during puppies and stabilize in adult dogs [34]. In addition, no difference on the digestibility of crude

236 protein was confirmed between weaning dogs or adult dogs and senior dogs [7,17,36]. However, the

237 decrease in the number of proliferative cells in the intestinal epithelium with age implies a decrease in

238 cellular turnover and may lead to a low adaptability to changes in the digestive environment [43].

239 Additionally, increased fiber fermentation by gut microbiota can increase microbial nitrogen excretion,

240 which may decrease the ATTD of crude protein [36,44].

241 In this study, the ATTD of crude fiber was lower in puppies than in adult or senior dogs. Since

242 fiber digestion occurs primarily through microbial fermentation in the large intestine, the difference may

243 be related to the development of the gut microbial ecosystem. Microbial diversity is a complex interaction

244   involving genetics, breed, birth type, and diet, which collectively compose the microbial community [15].

245   While previous studies have shown inconsistent results in age-related changes on microbial diversity [45–

246   47], it is generally accepted that the composition of gut microbiota undergoes rapid restructuring during

247   early life and reaches a relatively stable state by one year of age [46,48]. Thus, this microbial development

248   in adult and senior dogs may contribute to more efficient fermentation and subsequent short-chain fatty

249   acids (SCFA) production from dietary fiber. Furthermore, the similar ATTD of crude fiber between adult

250   and senior groups suggests that fiber digestibility is well maintained with age. This may be due to the low

251   non-starch polysaccharides and anti-nutritional factor contents of brown rice, which was used as the main

252   source in the experimental diet [49] and is supported by the results of improved digestibility reported in a

253   previous study [50,51].

254   The ATTD of ether extract and NFE was lower in puppies than in adult or senior beagles. These

255   results suggest that immaturity of digestive enzyme function in early life may contribute to reduced nutrient

256   utilization, which is consistent with previous findings indicating that digestibility values stabilize or

257   increase with age [7,13,36,37]. In addition, although the results of calorie-based DFO predicted a difference

258   in energy utilization between adult and senior dogs, the digestibility of energy-related nutrients did not

259   differ between the two groups. This suggests that there may be no difference in energy utilization for

260   maintenance between adult and senior dogs under normal physiological conditions. Compared with the

261   ATTD of crude ash, the ATTD of calcium and phosphorus showed opposite results with age. This suggests

262   that the metabolic utilization of minerals may differ across the life cycle of beagles. Ash is a broad indicator

263   of minerals and may indicate increased utilization with growth and development. On the other hand, the

264   improved or maintained ATTD of calcium and phosphorus with age may be related to morphological and

265   functional changes in the intestine of dogs [43,52]. In addition, it can be assumed that passive utilization is

266   enhanced due to an increase in mineral solubility by changing gut pH through the production of SCFA

267   following dietary fiber fermentation in the gut. Furthermore, since the calcium content in the food affects

268   the digestibility of phosphorus [53] formulation of food reflecting nutritional metabolic characteristics

269   should be considered. The ATTD of amino acids did not differ between age groups, which may have

270   induced changes in the amino acid profile due to contributions from the gut microbiota [38]. Therefore, the

271   evaluation of apparent ileal digestibility is also considered to evaluate the bioavailability of amino acids

272   [2,54]. Collectively, our nutrient digestibility results revealed differences by age, suggesting the need for

273   appropriate nutrient supply through diet composition according to developmental stage.

274   The CBC and serum biochemical profiling can objectively and specifically evaluate physiological

275   status, and screening or monitoring through the test is important in health management [55]. WBC play an

276   important role in response to inflammation, disease, and infection, and changes in their numbers indicate

277   health status. In this study, age-related differences were observed in the numbers of WBC, including

278   neutrophil, lymphocyte, and monocyte, suggesting dynamic changes in the immune system across life

279   stages. The results of WBC differentiation by age are similar to those of previous studies [16,18,56],

280   indicating changes in immunosenescence characteristics due to aging. It is possible that aging was

281   accompanied by inflammaging, a state of low-grade, chronic, and systemic inflammation [6,18]. In addition,

282   changes in WBC indices in senior beagles suggest a possible disruption of immune balance, which may

283   also be related to decreased oxidative stress response ability and susceptibility to infection. These

284   immunological changes are important considerations when developing health management and nutritional

285   intervention strategies for older dogs. In red blood cell indices, MCHC level was higher or trended higher

286   in adult beagles than in puppies or senior beagles during the study. On the other hand, MCV and MCH were

287   higher in the puppy group than in the adult or senior groups, but RDW and MPV were higher in the senior

288   group than in the puppy or adult groups. These results suggest an increase in erythrocyte heterogeneity due

289   to age-related development of immature red blood cells combined with the effects of inflammation or iron

290   deficiency [16,57]. Therefore, it is necessary to understand the physiological changes related to bone

291   marrow production and hematopoiesis by life cycle of dogs and provide appropriate nutritional management,

292   especially in senior dogs, as they can lead to chronic inflammation or signs of malnutrition.

293   In serum biochemistry, we observed age-related differences were observed in indicators reflecting

294   nutritional metabolism and tissue functions. Throughout the study, senior beagles consistently exhibited

295   lower concentrations of albumin, glucose, and creatinine in serum than puppies and adult beagles. On the

296   other hand, puppies had higher serum ALP level than adult and senior beagles. These findings suggest

297   changes in systemic metabolism associated with aging, involving the liver, kidney, and skeletal muscle

298   functions. The decline in serum albumin level with age may be due to reduced hepatic synthetic capacity

299   as well as renal and/or intestinal losses, as previously reported in aging dogs [58,59]. In addition, the

300   decrease in serum glucose level has been reported to be due to decreased hepatic glycogen storage function

301   or decreased functional liver mass [16,60]. Serum creatinine level may indicate changes in muscle mass or

302   renal function [61,62]. In addition, creatinine, a byproduct of muscle metabolism, may be present at lower

303   concentrations in young animals due to lower muscle mass [7]. In contrast, a previous study revealed a

304   positive correlation between serum creatinine and lean body mass [63]. It has been reported that an increase

305   in serum ALP level is an enzyme associated with the physiological response to bone growth, and that levels

306   were high during the bone growth period and decreased with age [7,62]. In this regard, puppies also have

307   higher serum calcium levels, reflecting active regulation of calcium metabolism during the growth process.

308   At the end of the experiment, puppies showed higher level of BUN than adult or senior dogs. Because BUN

309   is a product of protein metabolism, including hepatic urea cycle and renal excretion [64], elevated level in

310   puppies may reflect higher dietary protein intake in puppies than adult or old dogs. Taken together, these

311   results suggest changes in basal metabolic rate and nutritional metabolic functions with aging, and

312   differences in food intake should also be considered.

313

314   **Conclusion**

315   Our findings highlight the importance of considering age as a major factor influencing nutritional

316   requirements, digestive efficiency, and systemic health physiology in Beagle dogs. Overall, optimal

317   nutritional feeding is required during the growth period to support development with high nutrient

318   availability, and with advancing age to address changes in digestive capacity, nutrient metabolism, and

319   systemic health. In addition, this study suggests the need for age-specific food formulations and health

320   screening or monitoring strategies to optimize the health maintenance of dogs throughout their lifespan.

321   Collectively, the current study is expected to contribute to the development of age-specific dietary strategies

322   and long-term health management of companion dogs.

323

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511

512 # Tables

513 **Table 1.** Composition of experimental food for Beagle dogs (as-fed basis)

Item	Basal diet
Ingredient, %	
Hydrolyzed chicken powder	35.00
Brown rice	32.65
Soy protein	15.00
Tapioca starch	5.00
Canola oil	3.00
Sweet pumpkin	2.00
Cabbage	2.00
Carrot	1.00
Monocalcium phosphate	1.80
Calcium carbonate	1.60
Vitamin-mineral premix[1]	0.50
Tocopherol	0.05
Salt	0.40
Total	100.00
Calculated composition	
Dry matter, %	91.09
Metabolizable energy[2], kcal/kg	3,707
Crude protein, %	40.17
Crude fat, %	6.23
Crude fiber, %	2.72
Calcium, %	0.79
Phosphorus, %	0.66
Crude ash, %	6.55
Nitrogen-free extract, %	35.42

514   [1]Vitamin and mineral premix supplied per kg of diets: 3,500 IU vitamin A; 250 IU vitamin D3; 25

515 mg vitamin E; 0.052 mg vitamin K; 2.8 mg vitamin B1(thiamine); 2.6 mg vitamin B2 (riboflavin); 2 mg

516 vitamin B6 (pyridoxine); 0.014 mg vitamin B12; 6 mg Cal-d-pantothenate; 30 mg niacin; 0.4 mg folic acid;

517 0.036 mg biotin; 1,000 mg taurine; 44 mg $FeSO_4$; 3.8 mg $MnSO_4$; 50 mg $ZnSO_4$; 7.5 mg $CuSO_4$; 0.18 mg

518 Na_2SeO_3; 0.9 mg $Ca(IO_3)_2$.

519   [2]Metabolizable energy (ME) was calculated follow equation; ME (kcal/kg) − ([CP × 3.5] + [EE ×

520 8.5] + [NFE × 3.5]) × 10.

521 **Table 2.** Comparison of nutritional composition of experimental foods with Association of American Feed Control Officials (AAFCO) guidelines (dry

522 matter-basis)

Item	AAFCO			Basal diet[1]
	Growth and reproduction Minimum	Adult maintenance minimum	Maximum	
Crude protein, %	22.50	18.00		38.82
Arginine. %	1.00	0.51		1.28
Histidine, %	0.44	0.19		0.44
Isoleucine, %	0.71	0.38		0.84
Leucine, %	1.29	0.68		1.41
Lysine, %	0.90	0.63		1.15
Methionine, %	0.35	0.33		0.37
Methionine-cysteine, %	0.70	0.65		0.83
Phenylalanine, %	1.30	0.74		0.87
Threonine, %	1.04	0.48		1.06
Tryptophan, %	0.20	0.16		0.31
Valine, %	0.68	0.49		0.76
Crude fat, %	8.50	5.50		6.65
Calcium, %	1.20	0.50	2.50[2]	0.78
Phosphorus, %	1.00	0.40	1.60	0.65
Calcium:phosphorus	1:1	1:1	2:1	1.2:1

523 [1]Analyzed composition.

524 [2]The maximum calcium content level on a dry matter basis for other life stages including non-large size, and for large size dogs (those weighing

525 70 pounds or more as mature), the maximum is 1.8% [19].

526 **Table 3.** Age-related changes in growth and fecal parameters of Beagle dogs[1]

Item[2]	Puppy	Adult	Senior	SEM	*p*-value
Body size					
Initial body weight, kg	6.21[b]	8.16[a]	6.95[ab]	0.42	0.016
Final body weight, kg	6.94	8.23	6.91	0.47	0.104
Body weight change, g/d	72.50[a]	7.17[b]	-3.83[b]	11.54	< 0.001
Initial body condition score	5.50	4.50	5.58	0.58	0.369
Final body condition score	5.00	4.67	4.58	0.37	0.711
Food intake					
Daily food intake, g/d (as-is)	210.33[a]	172.33[b]	121.53[c]	6.54	< 0.001
Daily food intake, g/d (DM basis)	191.60[a]	156.99[b]	110.71[c]	5.95	< 0.001
Daily food intake, kcal/d	932.52[a]	764.04[b]	538.81[c]	28.98	< 0.001
Feces					
Daily fecal output, g/d (as-is)	63.75[a]	44.80[b]	31.76[c]	2.16	< 0.001
Daily fecal output, g/d (DM basis)	20.98[a]	16.11[b]	12.32[c]	1.01	< 0.001
Daily fecal output, kcal/d	66.27[a]	49.64[b]	39.82[b]	3.41	< 0.001
Fecal score	3.07	2.73	2.63	0.15	0.123
Fecal moisture, %	67.12[a]	63.97[ab]	61.35[b]	1.11	0.008
Diarrhea frequency, %	27.08[a]	8.33[b]	6.25[b]		0.022
Hard dry feces frequency, %	0.00	0.00	4.17		0.143

527   [1]Each value is the mean of 6 replicates (one dog per cage).

528   [2]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old; BW, body weight; ADG,

529 average daily gain; ADFI, average daily food intake; FCR, food conversion ratio; BCS, body condition

530 score; Diarrhea frequency = (number of fecal score ≥ 3.5 / number of cage days) × 100; Hard dry feces

531 frequency = (number of fecal score < 2.0 / number of cage days) × 100.

532   [a–c]Means with different superscript letters within a row are different ($p < 0.05$).

533

534

535 **Table 4.** Age-related changes in apparent total tract nutrient digestibility of Beagle dogs[1]

Item[2]	Puppy	Adult	Senior	SEM	*p*-value
Dry matter, %	89.07	89.76	88.95	0.39	0.323
Organic matter, %	95.49	96.10	95.27	0.32	0.192
Gross energy, %	92.90	93.51	92.68	0.37	0.289
Crude protein, %	92.39[a]	91.77[a]	89.99[b]	0.35	0.001
Ether extract, %	82.66[b]	85.28[a]	85.53[a]	0.57	0.005
Crude fiber, %	79.82[b]	83.57[a]	83.80[a]	0.60	< 0.001
Crude ash, %	64.58[x]	61.76[xy]	58.87[y]	1.66	0.082
Nitrogen-free extract, %	90.51[b]	92.42[a]	92.52[a]	0.34	0.001
Calcium, %	82.49[c]	84.45[b]	86.47[a]	0.62	0.002
Phosphorus, %	80.27[b]	85.01[a]	85.35[a]	0.64	< 0.001

536 [1]Each value is the mean of 6 replicates (one dog per cage).

537 [2]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old.

538 [a–c]Means with different superscript letters within a row are different ($p < 0.05$).

539 [x,y]Means with different superscript letters within a row are different ($p < 0.10$).

540

541 **Table 5.** Age-related changes in apparent total tract amino acid digestibility of Beagle dogs[1]

Item[2]	Puppy	Adult	Senior	SEM	*p*-value
Indispensable amino acids					
Arginine	97.78	97.93	97.78	0.09	0.462
Histidine	97.63	97.86	97.73	0.11	0.339
Isoleucine	97.68	97.83	97.68	0.09	0.443
Leucine	97.68	97.83	97.67	0.09	0.416
Lysine	97.77	97.91	97.76	0.09	0.456
Methionine	97.69	97.85	97.72	0.13	0.671
Phenylalanine	97.70	97.85	97.71	0.09	0.450
Threonine	97.73	97.88	97.73	0.09	0.428
Tryptophan	97.59	97.75	97.63	0.15	0.750
Valine	97.62	97.77	97.62	0.10	0.467
Dispensable amino acids					
Alanine	97.53	97.71	97.55	0.16	0.693
Aspartic acid	97.68	97.83	97.67	0.09	0.386
Cysteine	97.40	97.57	97.41	0.12	0.548
Glutamic acid	97.70	97.85	97.69	0.08	0.357
Glycine	97.67	97.82	97.67	0.09	0.426
Proline	97.74	97.89	97.74	0.09	0.447
Serine	97.68	97.83	97.68	0.11	0.547
Tyrosine	98.08	98.22	98.12	0.11	0.681

542   [1]Each value is the mean of 6 replicates (one dog per cage).

543   [2]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old.

544

545   **Table 6.** Age-related changes in hematological profile of Beagle dogs[1]

Item[2]	Puppy	Adult	Senior	SEM	p-value
Day 1					
White blood cell, $\times 10^3/\mu L$	10.70[a]	7.56[b]	7.60[b]	0.83	0.026
Neutrophil, $\times 10^3/\mu L$	7.56[a]	5.14[b]	5.07[b]	0.70	0.040
Lymphocyte, $\times 10^3/\mu L$	2.01	1.70	1.64	0.19	0.354
Monocyte, $\times 10^3/\mu L$	0.51[a]	0.31[b]	0.27[b]	0.03	< 0.001
Eosinophil, $\times 10^3/\mu L$	0.53	0.39	0.56	0.12	0.531
Neutrophil, %	69.77	67.81	66.46	2.29	0.599
Lymphocyte, %	19.22	22.49	21.78	1.78	0.415
Monocyte, %	4.94	4.25	3.85	0.53	0.360
Eosinophil, %	5.14	5.15	7.12	1.15	0.400
Red blood cell, $\times 10^6/\mu L$	6.24	6.84	6.13	0.26	0.157
Hemoglobin, g/dL	14.78	16.07	13.93	0.67	0.109
Hematocrit, %	41.37	44.12	39.72	1.79	0.245
MCV fL	66.37	64.55	64.72	0.99	0.381
MCH, pg	23.67	23.53	22.67	0.41	0.202
MCHC, g/dL	35.68[b]	36.43[a]	35.05[c]	0.20	0.001
RDW-CV, %	13.32	12.87	13.37	0.28	0.413
RDW-SD, fL	34.55	32.48	33.42	0.63	0.102
Platelet, $\times 10^3/\mu L$	268.83	325.17	132.67	64.59	0.130
MPV, fL	9.50	9.98	8.97	0.47	0.336
Day 10					
White blood cell, $\times 10^3/\mu L$	8.71[x]	7.62[xy]	5.89[y]	0.78	0.062
Neutrophil, $\times 10^3/\mu L$	6.29[x]	5.97[xy]	4.12[y]	0.71	0.096
Lymphocyte, $\times 10^3/\mu L$	1.76[a]	1.12[b]	1.32[ab]	0.17	0.049
Monocyte, $\times 10^3/\mu L$	0.50	0.40	0.34	0.06	0.213
Eosinophil, $\times 10^3/\mu L$	0.08	0.10	0.09	0.02	0.755
Neutrophil, %	72.10[xy]	77.58[x]	69.62[y]	2.31	0.074
Lymphocyte, %	20.24	15.55	22.69	2.30	0.117
Monocyte, %	5.80	5.19	5.99	0.71	0.715
Eosinophil, %	0.87	1.29	1.47	0.27	0.289
Red blood cells, $\times 10^6/\mu L$	6.63	7.00	6.64	0.19	0.308
Hemoglobin, g/dL	16.07	16.28	15.10	0.39	0.103
Hematocrit, %	45.58	45.75	43.43	1.19	0.334

MCV fL	68.80[a]	65.48[b]	65.40[b]	0.99	0.046
MCH, pg	24.27[a]	23.32[ab]	22.72[b]	0.34	0.019
MCHC, g/dL	35.35[xy]	35.60[x]	34.73[y]	0.26	0.080
RDW-CV, %	13.08[b]	12.90[b]	14.23[a]	0.33	0.024
RDW-SD, fL	34.92[ab]	32.92[b]	35.87[a]	0.70	0.027
Platelet, $\times 10^3/\mu L$	218.50	169.00	146.33	46.74	0.550
MPV, fL	9.10[b]	10.40[a]	10.18[a]	0.31	0.020

546   [1]Each value is the mean of 6 replicates (one dog per cage).

547   [2]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old; MPV, mean platelet

548 volume; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular

549 hemoglobin concentration; RDW-CV, red blood cell distribution width-coefficient of variation; RDW-SD,

550 red blood cell distribution width-standard deviation.

551   [a–c]Means with different superscript letters within a row are different ($p < 0.05$).

552   [x,y]Means with different superscript letters within a row are different ($p < 0.10$).

553

554 **Table 7.** Age-related changes in serum biochemical profile of Beagle dogs[1]

Item[2]	Puppy	Adult	Senior	SEM	*p*-value
Day 1					
Total protein, g/dL	5.15	5.15	5.17	0.03	0.923
Albumin, g/dL	3.57[a]	3.38[a]	2.82[b]	0.17	0.017
Glucose, mg/dL	108.50[a]	101.00[a]	94.17[b]	3.50	0.036
Triglyceride, mg/dL	35.50[y]	36.00[xy]	46.00[x]	3.47	0.085
Total cholesterol, mg/dL	142.50	174.83	165.67	14.75	0.308
Creatinine, mg/dL	0.34[a]	0.34[a]	0.20[b]	0.03	0.007
Blood urea nitrogen, mg/dL	16.77[y]	25.44[x]	21.22[xy]	2.44	0.072
Calcium, mg/dL	9.71	8.79	8.48	0.45	0.164
Alkaline phosphatase, U/L	95.33[a]	34.00[b]	47.50[b]	15.44	0.032
Aspartate aminotransferase, U/L	50.00	28.50	33.00	9.35	0.261
Alanine aminotransferase, U/L	37.40	32.83	26.00	3.41	0.108
Day 10					
Total protein, g/dL	5.83	4.35	5.73	0.99	0.513
Albumin, g/dL	4.13[a]	3.92[a]	3.42[b]	0.09	< 0.001
Glucose, mg/dL	109.00[a]	103.50[a]	93.17[b]	3.17	0.010
Triglyceride, mg/dL	40.50	41.33	41.17	3.45	0.984
Total cholesterol, mg/dL	124.50	162.17	148.83	13.07	0.152
Creatinine, mg/dL	0.30[a]	0.33[a]	0.23[b]	0.02	0.005
Blood urea nitrogen, mg/dL	40.18[a]	30.98[b]	26.12[b]	2.00	< 0.001
Calcium, mg/dL	10.76[a]	9.64[b]	9.72[b]	0.15	< 0.001
Alkaline phosphatase, U/L	70.50[a]	26.50[b]	36.00[b]	4.54	< 0.001
Aspartate aminotransferase, U/L	36.83	40.83	41.67	5.37	0.796
Alanine aminotransferase, U/L	33.80	34.33	39.00	3.49	0.590

555 [1]Each value is the mean of 6 replicates (one dog per cage).

556 [2]Puppy, under 1 year old; Adult, 1–7 years old; Senior, over 7 years old.

557 [a,b]Means with different superscript letters within a row are different ($p < 0.05$).

558 [x,y]Means with different superscript letters within a row are different ($p < 0.10$).